University of Glasgow

Form 1: Proposal and Risk Assessment for Work with Genetically Modified Microorganisms and or Animal Cells Excluding Use of Viruses

A GM risk assessment is required for the possession or use of genetically modified organisms. Please complete this form by computer and send it as an email attachment to your GM Biological Safety Officer (GMBSO) to submit it to your GM Safety Committee (GMSC). GMBSO will advise Principal Investigators on all aspects of GM risk assessment and HSE notification. Guidance on completing this form is provided on the Health and Safety section of the CMVLS website and GM Risk Assessment section of the SEPS website.

Title of project	Glasgow University 2015 iGEM team project: UVA light controlled bioluminescence		
GM reference	[ENTER DETAILS HERE]		
Principal investigator	Dr Sean Colloms		
School / Institute	The University of Glasgow		
Date of application	24/06/2015		
Location of work (Building & room numbers)	Bower Building, Wilkins Teaching Laboratory		
Connected programme (only for Class 2 or 3)	No	**If Yes, and not parent, give parent GM reference**	
If relevant, provide the Biological Services risk assessment serial number			

Section 1 Project

1.1: Brief description of the project, including the methods to be used and the purpose of the genetic modification (Preferably no more than 500 words unless the work is very complex)

The project is Glasgow University's 2015 entry to the iGEM (International Genetically Engineered Machine) competition. The competition involves the engineering of bacteria using "biobricks" - genetic blocks that can be arranged in a modular fashion.

The final aim of the project is to create a system in *E. coli* where a lack of UVA light exposure leads to activation of bioluminescence. In our hypothetical real world application the system could be utilised in containers as night-time lamps or road markings.

The final constructed system of the project will be made up by integrating three systems originating from three different organisms: A UVA sensor system (from *Synechocystis* sp. PCC 6803), a regulated promoter/repressor system (from either *Pseudomonas protogens* or *putida* S12) and a bioluminescence generation system (from *Aliivibrio fischeri).*

UVA Sensor: We will isolate and express the two-component *Synechocystis* UVA sensor system genes in *E. coli*, along with the sensor's corresponding promoter, and test by inserting the promoter upstream of a reporter (GFP). The sensor system functions by UVA light activation of a DNA binding domain, which will then bind the promoter region upstream of the GFP gene, leading to GFP fluorescence, proving the system from *Synechocystis* can work in *E. coli*. If the sensor system works in *E. coli* then we will perform characterising experiments such as quantifying the strength of transcription from the regulated promoter, and then use the UVA regulated promoter to control expression of the pseudomonas repressor described below.

Repressor systems: We propose to isolate two new transcription regulators and, PhlF and SrpR from *Pseudomonas*, and the promoters they regulate, to turn them into biobricks for the iGEM registry and utilise one of them to regulate bioluminescence in response to UVA. Use of a repressor between the UV sensor and the bioluminescence genes "inverts" the signal, allowing us to switch bioluminescence on in the absence of UV. We wish to perform characterising experiments such as inserting the new Repressors/Promoters upstream of the gene for GFP and compare to other biobrick repressor/promoter systems to check for cross activity between the systems.

Bioluminescence system: We will use the Lux operon of *Aliivibrio fischeri* to make our *E. coli* glow conditionally. The Lux operon has been well characterised but we hope to perform experiments where we separate and alter the expression levels of the individual components of the biosynthesis pathway in order to maximise luminescence intensity and duration for our system.

The methods used will be standard molecular biology practices for plasmid construction including PCR, transformation into *E. coli* K12, restriction digests, ligations site-directed mutagenesis and so on.

1.2: Will you cultivate on a large scale (eg 10 or more litres per culture)	No

1.3: Host organism

Escherichia coli K-12

1.4: Vector system

Plasmids – biobrick vectors with p15A, pBR322, pSC101 origins and ampicillin, chloramphenicol, kanamycin antibiotic resistances, non mobilisable, non conjugative. (Specifically pSB1C3, pSB3K3, pSB4A5, pSB1A2, J61002, pSB6A1,pSB3C5, and pCR 2.1-TOPO)

1.5: Genetic material (eg origins, nature of genetic modifications and intended functions)

UVA Sensor: The two component UVA sensor system we wish to utilise will be isolated from the cyanobacterium *Synechocystis* sp. PCC 6803. These genes will be amplified by PCR from PCC6803 genomic DNA, or made by total DNA synthesis.

Repressor systems: We wish to isolate two repressor/promoter systems, PhlF and SrpR, to utilise as inverters in our UVA controlled bioluminescent system. The genes for these repressors originate from *Pseudomonas protogens* and *Pseudomonas putida* S12. Both repressor genes and promoters will be made synthetically by Integrated DNA Technologies.

Bioluminescence system: We will use the "Lux" bioluminescence system, which originates from the marine bacterium *Aliivibrio fischeri*. We may also express the Lumazine protein from *Photobacterium phosphoreum* that shifts the bioluminescent emittance wavelength further from green to blue. These genes will be obtained from the iGEM parts distribution.

GFP- Originally from the jellyfish *Aequorea victoria* – for testing and quantification of the UV sensor and the two Repressor/Promoter systems. Genes for GFP will be obtained from the iGEM parts distribution.

The genetic material will not be altered, except to modify expression levels by changing ribosome binding sites and promoters, and to remove certain restriction sites without changing the encoded protein sequence, in order to make them iGEM biobrick standard compatible.

Section 2 Risk Assessment

This section should include clear and explicit justification of any statements made about the risks and be supported as far as is reasonable by adequate evidence and a logical explanation. **Level of risk** may be estimated using the matrix given at the end of this form and then stating the risk as either Effectively zero, Low, Low / Medium, Medium or High.

2A Risks to Human Health

2A.1: Characteristics of the host and any hazards associated with it

The host is a debilitated strain of *E.coli* K-12. Non-pathogenic strains of *E. coli* usually populate almost all mammalian guts. The K-12 strain's characteristics are well documented, and it has a history of safe use in the GM field. It has limited capacity to colonise the mammalian gut, lacking some of the specific structures required for attachment. Even if attachment was successful, it does not possess any pathogenic properties. The strain we will use for many of of our experiments (DS941) is auxotrophic for six amino acids due to mutations in its genome, whereas the other strains (Top10 and DH5alpha) have a mutation in the *recA* gene and are therefore highly sensitive to UV irradiation. Therefore they do not survive well outside the laboratory environment. In addition, *E. coli* K12 is not believed to have any adverse effects on the environment, animals or plants.

Level of risk	**Effectively zero**

2A.2: Characteristics of the vector system and any hazards associated with it

The plasmids used are both non-mobilisable and non-conjugatable, which should remove the risks of undesirable plasmid transfer to other species. Little hazard is associated with the material transferred by the plasmids, regardless – the genetic material on the plasmids has not been engineered to produce chemicals or proteins potentially harmful to the environment, plants, animals or humans.

Level of risk	**Effectively zero**

2A.3: Source and characteristics of the inserted gene product and any hazards arising directly from

its use (including an estimation of the level of expression and biological activity of the recombinant gene product)

In *Synechocystis*, UirS protein senses UVA light and phosphorylates UirR protein into a DNA binding form, which binds to the promoter region of the following gene LsiR and activates transcription. In vivo, LsiR upregulation leads to a "negative phototactic" response where the cell moves away from the UVA source. The chromophore cofactor Phycocyanobillin (PCB) is also native to *Synechocystis* and is required for light sensation. PCB is synthesised from Heme by a two-gene pathway (ho1 and PcyA). The *Synechocystis* UVA sensor genes will be PCR amplified from a sample of genomic DNA sourced from another lab in the university, and the PCB synthesis genes will be sourced from the iGEM registry of parts. *Synechocystis* organism will not be used in the laboratory. *Synechocystis* PCC6803 is classified as a biosafety class 1 organism (ATCC 27184).

PhlF repressor originates from the plant-protecting soil organism *Pseudomonas protogens*, where it regulates expression of synthesis of the anti-microbial compound 2,4-Diacetylphloroglucinol (DAPG) that acts against plant pathogens. SrpR repressor originates from soil organism *Pseudomonas putida* S12 where it regulates expression of synthesis of a solvent tolerance efflux system. The genes encoding promoter sequences and repressor proteins for the respective systems are to be directly synthesised to order from the sequences provided by the original paper which designed and characterised them (*Genomic mining of prokaryotic repressors for orthogonal logic gates,* Stanton et al., 2014) If project time allows we may isolate DAPG from *Pseudomonas protogens* culture, but otherwise neither strain of *Pseudomonas* will be present in the laboratory. *Pseudomonas protogens* and *Pseudomonas putida* S12 are both classified as a biosafety class 1 organisms (ATCC 17386 and 700801 respectively).

The Lux operon consists of six genes (LuxA, B, C, D, E, G) from *Aliivibrio fischeri* that encode for the synthesis pathway of Tetradecanal aldehyde and the Luciferase enzyme which oxidises Tetradecanal to release light. We may also express the gene LumP from *Photobacterium phosphoreum* that encodes Lumazine chromophore protein that absorbs the blue-green bioluminescent light emission and re-emits it at a shorter bluer coloured wavelength. Both the Lux system and LumP genes will be isolated from iGEM biobrick distribution plasmids. Aliivibrio fischeri is a type 1 biosafety organism (ATCC 7744).

GFP – Originally from the jellyfish *Aequorea victoria.* It fluoresces green light when exposed to light in the blue/violet wavelength range. It has a history of safe use in a number of fields, and should not pose a risk to human health.

Level of risk	**Effectively zero**

2A.4: Hazards arising from the alteration of any existing pathogenic traits, if applicable

N/A

Level of risk	**N/A**

2A.5: Potential hazards of sequences within the GMM being transferred to related micro-organisms

The genes detailed above allow UV photoreception, Gene signal inversion, and bioluminescence. There are no harmful chemicals or proteins being produced.

Level of risk	**Effectively zero**

2A.6: The overall likelihood that, in the event of exposure, the GMM could cause harm to human health

Extremely low risk – as mentioned, the host has limited capacity for colonisation or survival outside of the laboratory. The genes we will introduce into the GMM are highly unlikely to be harmful.

Level of risk	**Effectively zero**

Assign the provisional containment level: (Delete as appropriate)	**1**

2B Risks to Environment

2B.1: What is the capacity of the GMM to survive, establish, disseminate with and or displace other organisms

The *E.coli* K-12 is a debilitated strain, posing little to no risk to human, plant or other animal health. The *E.coli*'s capacity to populate the mammalian gut is not present on this strain, and it will not survive long outside of a laboratory environment due to a lack of colonising ability, its requirement for six amino acids and/or its sensitivity to UV irradiation.

Level of risk	**Effectively zero**

2B.2 What is its ability to cause harm to animals

Unlike other strains of *E.coli*, K-12 does not populate the mammalian gut, lacking the proteins required for fixing to the intestinal walls. Being unable to colonise, it presents negligible pathogenic risk to animals.

Level of risk:	**Effectively zero**

2B.3: What is its ability to cause harm to plants

E.coli K-12 is not known to cause harm to plants. The genes we will be inserting into the GMM are harmless to plants.	
Level of risk	**Effectively zero**

2B.4: What is its ability to cause harm to other organisms

E.coli K-12 is not known to cause harm to other organisms. The genes we will be introducing into *E. coli* K12 will not make it produce any harmful proteins or chemicals so there is little chance that the modifications will allow *E. coli* K12 to harm other organisms.	
Level of risk	**Effectively zero**

2B.5: What is the potential for transfer of genetic material between the GMM and other organisms

The chance of transfer is very low – the plasmids are both non-conjugative and non-mobilizable	
Level of risk	**Effectively zero**

2B.6: Is there any hazard as a result of phenotypic or genetic instability

The genetic material and resulting structures/chemicals are not close in structure to any toxins, so even if there was some instability (and we don't anticipate this), the result is unlikely to be hazardous	
Level of risk	**Effectively zero**

2C Final Activity Class

Assign the class: (Delete as appropriate)	**1**

Section 3 Control Measures to be Used

3: Provide details of the control measures to be used to protect human health and the environment and the means by which their use and effectiveness will be monitored. This must include details of the inactivation procedures to be employed for waste contaminated with GMM, the expected degree of kill and any appropriate validation procedures

3.1: Containment level | 1

The containment level of the lab is designated 1, which is a suitable level for the type of organisms and genetic modifications being carried out. As stated previously, the non-pathogenic nature of the *E.coli* K-12 strain is well documented and is not in any doubt. In addition, none of the modifications being made are potentially toxic.

3.2: Controls

The following controls are in place to stop the transport of any organisms outside the laboratory:
Wearing labcoats inside the lab, and removing them when going outside
Wearing gloves when handling material, and regularly checking them for holes.
Magnetic doors – with alarms if the door is left open for too long.
Aseptic techniques – general lab safety: including hand washing before and after working with the organisms and leaving the laboratory.
Disinfection/autoclaving of any culture of GMM or any apparatus that has come in contact with GMM prior to washing or disposal.

3.3: Inactivation of genetically modified organisms

Disinfection
Any contaminated equipment/worktop space is disinfected with the detergent "Chemigene", which is distributed throughout the lab in spray bottles (2% solution). Accurate degree of kill could be not obtained, but the chemical is widely used in the laboratory, with a history of effective use against the *E.coli K-12* strain.

The effective of Chemigene will be regularly tested by observing its effect on a culture of bacteria – it should stop all growth and kill all cells which will be tested by plating treated cultures.
The Chemigene is expected to have a lab diluted shelf life of 6 months – they will be refilled before the beginning of the 10 week project to maintain maximum effectiveness.
Equipment is then autoclaved.

Autoclaving
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile.
Or
All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121ºC for 20 min).

3.4: Waste disposal

Plastics (pipette tips, etc), are autoclaved as detailed above.
Plates containing bacteria are placed into a biohazard bag, and autoclaved.

Section 4 Emergency Planning

4:	**Are there risks to human health and safety or to the environment that require an emergency plan for action in case of accidental release**	No
If Yes, provide full details of the plan		

Section 5 Declaration and Approval

Declaration	**I declare that this work will be conducted in accordance with University rules, practices and requirements on GM procedures. If at any stage there is any indication that hazards or risks could be significantly higher than originally assessed or that changes to controls are needed then the work will cease until the risk assessment has been revised and approval granted from the GM Safety Committee.**
Principal investigator	
As the principal investigator for this GM project you have a legal responsibility to ensure that all those involved or working on the project have an appropriate level of training and expertise to enable safe working. This includes ensuring that they read and understand this risk assessment and that all procedures they undertake including the control measures are in strict accordance with those approved for the project. To ensure the latter you are advised to check for compliance with procedures and make an appropriate record to be kept as part of the project file.	
Sign and date	 24th June 2015
Declaration	**I declare that this risk assessment has been scrutinised and approved by the GM Safety Committee.**
To be signed by the GMBSO or in the event they are the principal investigator by another member of the GM Safety Committee.	
GMBSO	
Sign and date	

Risk Estimation Matrix

Consequence of hazard	**Likelihood of hazard**			
	High	**Medium**	**Low**	**Negligible**
Severe	High	High	Medium	Effectively zero
Modest	High	Medium	Medium / Low	Effectively zero
Minor	Medium / Low	Low	Low	Effectively zero
Negligible	Effectively zero	Effectively zero	Effectively zero	Effectively zero